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This study demonstrates that both the clinical sensitivity and specificity of the Cervista HPV HR test for high-risk human papillomavirus (HPV) detection are not inferior to those of the Hybrid Capture 2 (HC2) test. The intra- and interlaboratory reproducibilities of Cervista were 92.0% (kappa, 0.83) and 90.4% (kappa, 0.80), respectively. The Cervista HPV HR test fulfills all the international HPV test requirements for cervical primary screening purposes.

It is well established that cervical cancer is caused by a persistent infection of cervical epithelial cells by any of the ~14 genotypes of high-risk human papillomavirus (hrHPV). This knowledge prompted the development of *in vitro* diagnostic tests for hrHPV testing in clinical specimens. Generally, these tests have a high sensitivity and high negative predictive value, making them potentially valuable tools for primary screening strategies. Systematic reviews have shown that primary screening using hrHPV testing has a higher sensitivity than that of cytology for detecting cervical intraepithelial neoplasia grade 2 or higher (CIN2+) (1, 2). Although many current international guidelines limit HPV testing to the triage of borderline lesions and to post-CIN follow-up, it is believed that in the near future, HPV testing will be included in most European official guidelines as a viable strategy for primary screening (3). In line with the international guidelines for HPV DNA testing in primary cervical cancer screening in women ≥30 years described by Meijer et al. (4), the recently updated guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) emphasize the importance of using a validated HPV test, i.e., an HPV test that has proven acceptable reproducibility, clinical sensitivity, specificity, and positive and negative predictive values for cervical cancer screening of CIN2+ lesions (5).

The Cervista HPV HR test (Hologic, Inc., Madison, WI) was the second hrHPV assay approved by the FDA in 2009, 10 years after the approval of the Hybrid Capture 2 hrHPV DNA (HC2) test. The Cervista HPV HR assay uses Invader chemistry, a signal amplification method, to qualitatively detect specific nucleic acid sequences of 14 hrHPV types (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68), as described previously (6), and it utilizes a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal. Both types of reactions rely on oligonucleotide hybridization, invasive structure formation, and cleavage by the Cleavase enzyme. Interpretation of the HPV results was done in accordance with the Cervista HPV HR test package insert (7). The Cervista test has a few advantages over the HC2 test: it detects the same hrHPV types as the HC2 test plus HPV66, it requires half the sample volume of that of the HC2 test, it includes the human histone 2 gene as an internal control for sample adequacy, it demonstrates no cross-reactivity with common nononcogenic HPV types, and it has a shorter processing time. In comparative studies, the Cervista HPV HR test shows sensitivity and specificity results that are similar to those of the HC2 test (8–17), but studies comparing both assays on the same samples in a population-based screening setting are limited (9–11). Here, we evaluated the Cervista HPV HR test according the international guidelines for HPV DNA testing in primary cervical cancer screening in women ≥30 years by performing the validation process in strict accordance with that recommended by Meijer et al. (4).

The clinical performance of the Cervista HPV HR test was assessed relative to that of the HC2 test using data from the SHENCCAST II study, a large cohort of screening participants originally screened by cotesting using ThinPrep cytology and hrHPV testing, applying both the Cervista HR HPV and HC2 HPV tests, all performed on the same sample. A detailed description of the SHENCCAST II study, a multisite, population-based, and cross-sectional study conducted in Guangdong Province in China, which enrolled approximately 10,000 women, 25 to 59 years old, was reported previously (10). To calculate the relative clinical specificity and sensitivity, 7,218 samples without CIN2+ lesions and 109 samples with CIN2+ lesions, respectively, were used for a noninferiority analysis of the Cervista assay versus the HC2 assay. The overall clinical specificities of the HC2 and the Cervista assays in 7,218 women aged ≥30 years without CIN2+ (controls) were similar, at 89% (95% confidence interval [CI], 88.0 to 89.5) and 91% (95% CI, 90.5 to 91.8), respectively (Table 1). To calculate the relative sensitivity on a representative population-based screening cohort, 78 randomly selected samples with abnormal cytology (≥atypical cells of undetermined significance [ASCUS]) and 31 samples with normal cytology (negative for in-

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TABLE 1 Comparison of the Cervista HPV HR and HC2 test findings among 7,327 scrapings collected in the multisite, population-based, and cross-sectional SHENCCAST II study

Sample type (CIN score)	Cervista HPV HR result	HC2 HPV test result (no. of samples)		Total no. of samples
		Positive	Negative	
Controls (<2)	Positive	518	119	637
	Negative	291	6,290	6,581[a]
	Total	809	6,409[b]	7,218
Cases (2+)	Positive	97	0	97[c]
	Negative	5	7	12
	Total	102[d]	7	109

[a] The overall clinical specificity for the Cervista HPV HR test was 91% (95% CI, 90.5 to 91.8).
[b] The overall clinical specificity for the HC2 test was 89% (95% CI, 88.0 to 89.5).
[c] The overall clinical sensitivity for the Cervista HPV HR test was 89% (95% CI, 81.6 to 94.2).
[d] The overall clinical sensitivity for the HC2 test was 94% (95% CI, 87.2 to 97.4).

TABLE 2 Results of the intralaboratory reproducibility and interlaboratory agreement of the Cervista HPV HR test among 510 samples[a]

Test	Cervista HPV HR test result	No. with UMCG test 1 result:			Total no.
		Positive	Negative	Low gDNA[b]	
UMCG test 2 results	Positive	174	17	0	191
	Negative	24	293	1	318
	Low gDNA	0	0	1	1
	Total	198	310	2	510
Bruges test results	Positive	179	35	0	214
	Negative	12	281	1	294
	Low gDNA	0	1	1	2
	Total	191	317	2	510

[a] The agreement between the two test results (UMCG test 1 and UMCG test 2) was 92.0% (lower bound of 95% CI, 89.7%; kappa, 0.83; $P < 0.001$). The agreement between the two independent laboratories (UMCG test 1 compared with the Bruges test) was 90.4% (lower bound of 95% CI, 88.4%; kappa, 0.80; $P < 0.001$).
[b] gDNA, genomic DNA.

traepithelial lesion or malignancy [NILM]), all with histologically proven CIN2+ lesions (45 with CIN2, 61 with CIN3, and 3 with carcinoma), were selected (cases) from the SHENCCAST II data set. The overall clinical sensitivities of the HC2 and Cervista assays for detecting CIN2+ in women age ≥30 years were 94% (95% CI, 87.2 to 97.4) and 89% (95% CI, 81.6 to 94.2), respectively (Table 1). The noninferiority of the relative sensitivity and specificity of the Cervista HPV HR test versus the HC2 test was confirmed, as the null hypothesis of inferiority was rejected ($t = 17.73$, $P < 0.0001$ for specificity; $t = 1.76$ and $P = 0.043$ for sensitivity). Therefore, the Cervista HPV HR test met the criterion of noninferiority set forth by the international guidelines, i.e., it had a clinical sensitivity not less than 90% of the sensitivity of the HC2 test and a clinical specificity not less than 98% of the specificity of the HC2 test for detecting CIN2+ in women age ≥30 years.

The intra- and interlaboratory reproducibilities of the Cervista assay were evaluated on 510 cervical scraping samples selected from women age 30 to 60 years participating in the routine national population-based cervical screening program in the Netherlands. A detailed description of the sample selection and analysis of the intra- and interlaboratory reproducibilities is reported elsewhere (13). These samples comprised 186 HC2-positive and 324 HC2-negative randomly selected scrapings (36% hrHPV positivity), according to the international guidelines for HPV DNA testing. To determine the intralaboratory reproducibility, all 510 samples were tested twice (University Medical Center Groningen [UMCG] test 1 and UMCG test 2) with the Cervista assay, according to the manufacturer's product insert (7), at a 1- to 3-week interval by the same experienced technician on the same Cervista system at the Department of Pathology of the University Medical Center Groningen (UMCG). The agreement between the two test results was 92.0% (lower bound of the 95% CI, 89.7%; kappa, 0.83; $P < 0.001$) (Table 2). For the interlaboratory agreement, an aliquot (2 ml of PreservCyt) of the same samples was sent to an independent reference laboratory in Bruges (Department of Pathology, AZ St. Jan Brugge-Oostende, Bruges, Belgium), which routinely uses the Cervista assay. All samples were randomly renumbered and provided to the reference laboratory without any cytology or hrHPV test results. The agreement between the two laboratories was 90.4% (lower bound of the 95% CI, 88.4%; kappa, 0.80; $P < 0.001$) (Table 2). Thus, the intra- and interlaboratory agreements of the Cervista assay met the requirement of having a lower bound of the 95% CI of >87% and a kappa value of >0.5.

The present study validates the Cervista HPV HR test for use in primary screening for the detection of CIN2+ lesions in women age ≥30 years, in accordance with the international guidelines for HPV DNA testing in primary screening, and it includes a determination of: (i) the noninferiority of the Cervista test to the reference HC2 HPV test and (ii) the intra- and interlaboratory reproducibilities of the Cervista assay. The Cervista test met the criteria for noninferiority to the HC2 test (noninferiority test) set forth in the international guidelines (4). Thus, the Cervista HPV HR test fulfills all the requirements of the international guidelines and can be considered formally validated for the use of primary cervical cancer screening in women of age ≥30 years. Recently, we found that the specificity of the Cervista HPV HR test could be even further improved when the standard second cutoff (default setting of the manufacturer) was adapted (13).

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